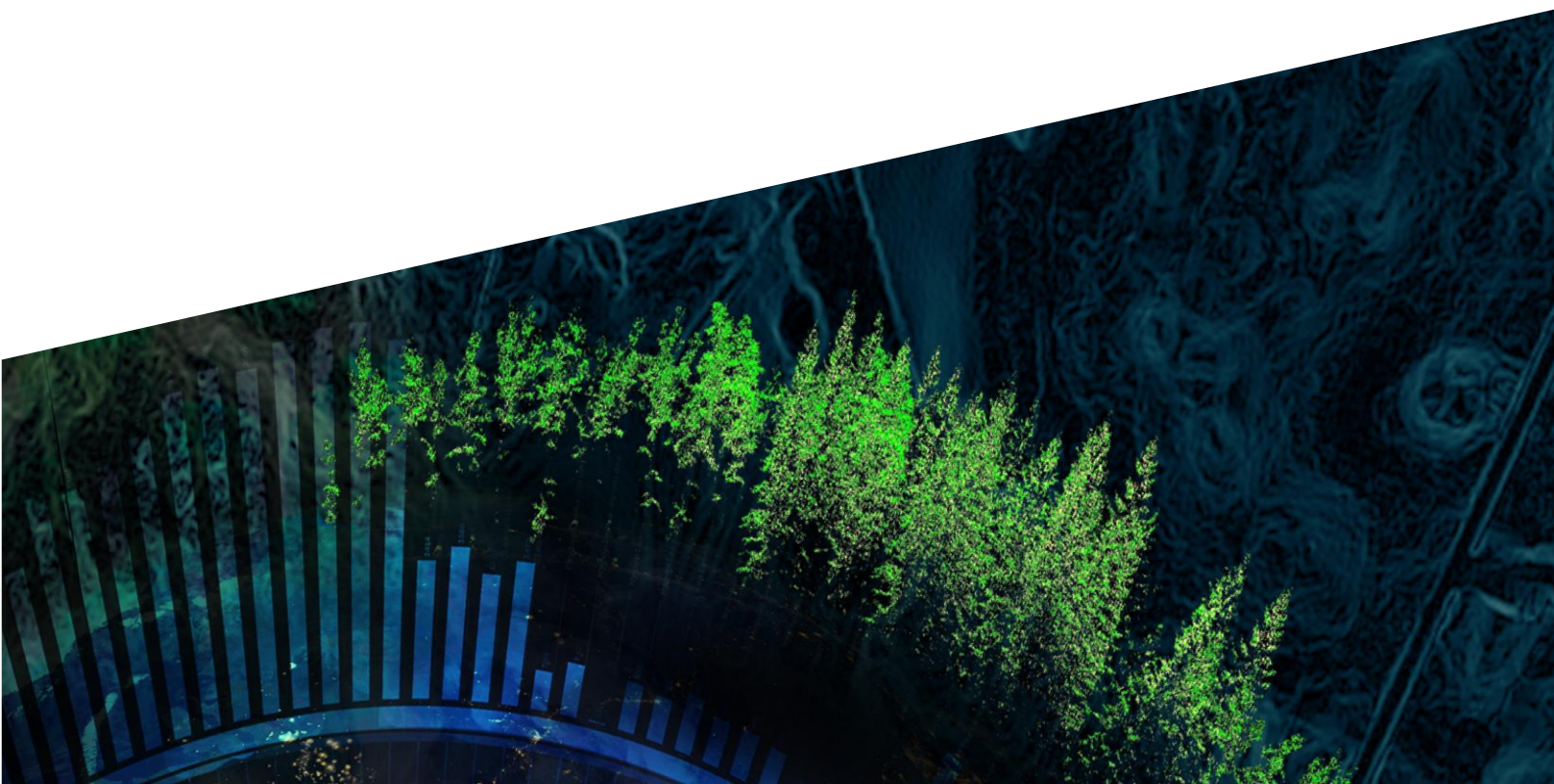




Genetic mapping on local Swedish chicken breeds

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Independent project: 30 hp
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
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European Master in Animal Breeding and Genetics (EMABG)
Uppsala 2021



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Credits: 30 hp
Level: Advanced, A2E
Course title: Independent project in Animal Science
Course code: EX0870
Programme/education: European Master in Animal Breeding and Genetics (EMABG)
Course coordinating dept: Department of Animals Breeding and Science

Place of publication: Uppsala
Year of publication: 2021

Keywords: chicken, genetic diversity, GWAS, plumage color, SNP, Swedish chicken breed

Swedish University of Agricultural Sciences
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Abstract

Today, genetic studies are gaining popularity around the world, especially in the developed world. The study of genetic diversity is the basis for genetic protection and future breed improvement. The current study aimed to assess the genetic diversity, genetic relationship, and to identify the genes affecting the plumage colors of eight Swedish chicken breeds. There are about 11 breeds of Swedish chickens in Sweden. The study breeds were Gotlandshöna, Öländsk dvärghöna, Kindahöna, Hedemorahöna, Skånsk blommehöna, Åsbohöna, Ölandhöna, and svarthöna chicken.

A total of 83 chickens were genotyped using a 62K SNP chip. The mean observed heterozygosity of the study breeds was 0.40 and the mean inbreeding coefficient (F) of the study breeds calculated from the discrepancy of observed and expected heterozygotes was -0.07. The mean F_{ST} of the study breeds was 0.36, which indicated that the Swedish chicken breeds were very diverse. The study breeds formed 3 main clusters in the multi-dimensional scaling (MDS) plot based on their genetic relationship, where most of the breeds were grouped in one of the main groups. Due to population structure, it was not possible to identify potential SNPs involved in plumage color variation.

To do GWAS for plumage color variability of Swedish chickens, the sample size must be much larger. The current study on genetic diversity may help to strengthen the genetic conservation program, such as, eliminating inbreeding and conducting additional molecular-based studies. Further research into plumage color variability should be done, by including many more individuals.

Keywords: chicken, genetic diversity, GWAS, plumage color, SNP, Swedish chicken breed

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List of abbreviations

Chr – Chromosome
F --- Inbreeding coefficient
FIS--- Individual heterozygosity relative to the subpopulation
FST – Fixation index
Geno ---Missing genotype rate
GWAS ---Genome-wide association study
HWE ---Hardy Weinberg Equilibrium
MAF ---Minor allele frequency
Mds ---Multi-dimensional scaling
Mind ---Missing genotype rate
Ne ---Effective population size
PCs --- principal components
PIC ---Polymorphism information content
QQ plot ---Quantile- Quantile plot
SNP ---Single nucleotide polymorphism
 λ ---genomic inflation factor

1. INTRODUCTION

1.1. Background

The ancestor of the domestic chickens (*Gallus gallus domesticus*) originated from the wild junglefowl (*G. gallus*) in Southeast Asia (Crawford, 1990; Moiseyeva et al., 2002). The genus *Gallus* had four main wild species, which are expected to be the ancestor of domestic chickens; red junglefowl (*G. gallus*), gray junglefowl (*G. sonnerati*), green junglefowl (*G. varius*), and Sri Lankan junglefowl (*G. lafayetii*). However, the genetic predisposition of each species to the modern chicken has not been well discovered (Dessie et al., 2012; Moiseyeva et al., 2002), but, based on the archaeological and molecular evidence, it is widely described that the red junglefowl is the main ancestral origin of domestic chicken.

Local chicken breeds make a significant economic contribution in many countries (Barua & Yoshimura, 2017; Kingori et al., 2010). They are highly adapted to the local environmental condition and require a minimum input for farming (Kaya and Yildiz, 2008). They are also selected by their nature and may have unique genetic differences. However, they have poor performance and less attention to genetic protection than other breeds (Blackburn, 2006; FAO, 2007). Most phenotypic and genetic studies have also focused on high-performing commercial chicken breeds (FAO, 2011; Granevitez et al., 2007).

The selection and breeding of some poultry began in the mid-19th century (FAO, 2007). The growing demand for improved breeds of chickens and the availability of modern technologies are increasing (Englund et al., 2014). A long-term breeding effort supports the improved breeds to dominate the current poultry production sector. Indigenous breeds are threatened by the commercialization of improved breeds. Poultry breeding supports the use of improved breeds and the replacement of indigenous breeds with improved breeds (FAO, 2011; Granevitez et al., 2007).

There are about eleven local Swedish chicken breeds in Sweden (Abebe et al., 2015). Before being rescued by the Swedish Local Chicken Association, the number of individuals of the breeds was very small (Englund et al., 2014). The association has maintained these chickens directly in the form of a gene bank in collaboration with its members and other partners (<http://www.kackel.se>) (Abebe et al., 2015). The individual chicken was originated from different areas of Sweden and may have been selected for a variety of adaptations. Although they pass

between a lot of bottle-neck, there are many phenotypic variabilities (Englund et al., 2014).

Previous studies on the genetic diversity of local Swedish chicken have reported a lower genetic diversity among breeds. A study on nine breeds using the mtDNA D-loop reported a limited mtDNA diversity between breeds with seven different haplotypes and most of the breeds had the same haplotype (Englund et al., 2014). A limited genetic difference between Swedish chicken breeds was also reported by Abebe and others (2015) using a 24K SNP chip. In all studies, relatively high molecular co-ancestry has been found, which may increase the rate of inbreeding in successive generations.

Recent advances in genetic markers provide many options for estimating genetic diversity among populations at the DNA level, rather than identifying species based on their phenotypic traits. Microsatellites are very numerous and are distributed evenly throughout the genome at high levels of mutations (Anmarkrud et al., 2008). they provide accurate information because they have a higher rate of occurrence of common alleles and have more polymorphism over the population (Maudet et al., 2002). Recently, a lot of researches have been done to assess the genetic diversity of chickens using microsatellite markers, and the results are a clear indicator of the usefulness of these markers for biodiversity research (Kaya & Yildiz, 2008; Ramadan et al., 2012; Wilkinson et al., 2012).

In poultry, plumage color is an economically important trait and has cultural value in many parts of the world (Huang et al., 2020). Plumage color may be used as a genetic marker to identify species, breeds, and breeding groups (Moisseva et al., 2012), as camouflage and sex symbol (Gluckman & Cardoso, 2010; Seddon et al., 2013; Wilkins et al., 2016). Feather formation is more complex than mammalian hair color (Yu et al., 2004). Many colors and patterns appear on a single feather.

Currently, only a few genes associated with plumage color have been identified (Makarova et al., 2019). There are many factors involved in expressing the color of a plumage (Kerje, 2003). The use of molecular genetic techniques can accurately identify genes responsible for phenotypic variability. This is because different colors can be controlled by the same gene, and different genes can produce the same color scheme. Identifying genes that regulate biological behavior provides direction for the genes of interest.

The present study focused on eight Swedish chicken breeds using the 62K SNP chip. The study breeds are Gotlandshöna chicken abbreviated to Gotl, Öländsk dvärghöna chicken abbreviated to ÖLDvärg, Kindahöna chicken abbreviated to Kinda, Hedemorahöna abbreviated to Hedem, Skånsk blommehöna chicken abbreviated to SkBlom, Äsbohöna chicken abbreviated to Äsbo, Ölandhöna chicken abbreviated to Öland, and Svarthöna chicken abbreviated to Svart.

1.2. Statement of Problem

Studies on the genetic diversity and relationship among breeds provide insights into the genetic similarities and differences between breeds (Abebe et al., 2015). Knowledge of the genetic diversity of the breed can be a source of information for future breed development, implementation of successful breeding programs, and future scientific research. Thus, a study on the genetic diversity of Swedish chicken breeds helps to strengthen the genetic conservation program of local Swedish chicken breeds, such as eliminating inbreeding and implementation of further Swedish chicken breeding research.

Besides, a genome-wide study on the phenotypic variability in Swedish chickens can serve as a baseline for further study of the molecular basis of plumage color formation. However, there is limited research on the genetic diversity of local Swedish chickens, and no previous studies have been done to identify the genes associated with plumage color. Therefore, it is important to study the genetic diversity, genetic relationship, and to identify the genes that cause the change in the plumage color of Swedish chicken breeds.

1.3. Objectives

1. To evaluate the genetic diversity of local Swedish chicken breeds
2. To evaluate the genetic relationship of local Swedish chicken breeds
3. To identify the genes involved in the plumage color variability of local Swedish chicken breeds

2. LITERATURE REVIEW

2.1. Genetic diversity within and between breeds

Heterozygosity levels are a reflection of genetic diversity within races (De et al., 2017). When the breed is under HWE, the genetic diversity is similar to the expected heterozygosity estimation. Lower heterozygosity indicates the occurrence of a higher rate of inbreeding (Abebe et al., 2015). The average observed and expected heterozygosity variability was due to evolutionary forces, such as random change of allelic frequency (Masatoshi, 1978). A low level of within-breed diversity can be related to small effective population size (N_e), inbreeding, genetic drift, null allele effect, and breeding practices, and lack of effective breeding strategies (Aubin-Horth et al., 2005; Dakin & Avise, 2004).

Heterozygosity deficiency may be due to the presence of homozygous alleles at the same locus (Dakin & Avis, 2004). The excess of homozygosity also resulted from the existence of null alleles. Keeping breeds in small populations and small isolated flocks results in the loss of heterozygosity for many generations (Young & Clark, 2000). Excess of homozygosity can lead to locus-specific heterozygosity deficit and a mismatch in well-known parent-to offspring relationships (Castro et al., 2004; Dakin & Avis, 2004; Wilkinson et al., 2012).

Various studies have examined the genetic diversity of Italian native chickens. A study by Viale and others (2017) reported the mean observed and expected heterozygosity varied from 0.124 to 0.244 and 0.132 to 0.300 respectively, using next-generation sequencing technologies. A study by Zanetti and others (2010) using 20 microsatellites markers, reported the mean observed and expected heterozygous of 0.35 and 0.33 respectively, and an average F_{ST} of 0.54. Cendron and others (2020) reported a low genetic diversity relative to commercial stocks, using Affymetrix 600 K Chicken SNP Array.

Numerous studies have examined the genetic diversity of Chinese native chickens. A study by Zhang and others (2018) using Illumina HiSeq 4000 sequencer, reported a significant genetic difference with the F_{ST} value ranged from 0.0046 to 0.1530. Another study by Zhang and others (2020) showed the pairwise F_{ST} estimates ranged from 0.03 to 0.27 using the 600K SNP chip. A study by Chen and others (2019) using a 600K SNP chip, reported a larger number of nucleotide diversity in China than European races and commercial species.

Historical processes, environmental factors, and life history, such as matting, can influence the genetic structure of the population (Gerlach & Musolf, 2000). A study by Malomane and others (2019) using high genomic resolution techniques reported that African chicken breeds (Egyptian, Sudanese, Ethiopian, and South African) shared with the Saudi Arabian and European gene pool. Egyptian chicken breeds have a higher genetic diversity among breeds (Eltanany et al., 2011).

PIC is one of the most important indicators of the quality of genetic markers. It measures the informativity and the usefulness of markers for linkage analysis (Guo & Elston, 1999). PIC for the co-dominant markers is ranged from 0 (monomorphic) to 1 (very informative) (Smith et al., 1997). According to (Dakin & Avise, 2004) $PIC > 0.5$ is more informative, $0.25 < PIC < 0.5$ is moderately informative and $PIC < 0.25$ is slightly informative. Using genetic markers is important in a variety of contexts, including admixture, gene flow, association mapping, and evolutionary history (Shriver et al., 1997, Bamshad et al., 2003; Ziv & Burchard, 2003). If the genetic markers are highly informative such studies would be more accurate for the inference of an individual's ancestry, while less informative markers are less sufficient to make an inference

Highly informative markers reduced the required amount of genotyping for genetic predisposition. PIC of a marker is related to the ability of the markers to detect the polymorphism between individuals, where the higher the polymorphism the higher its potential (Serrote et al., 2020). Various studies have evaluated the polymorphic information content (PIC) of different poultry breeds; Italian breed (0.54) (Zanetti et al., 2010), Egyptian breed (0.61) (Eltanany et al., 2011), southern Xinjiang chicken breed (0.79) (Azimu et al., 2018) and Swedish breed (0.56) (Abebe et al., 2015) studied using 24 microsatellite markers. PIC is a measure of informativity of genetic markers (Guo & Elston, 1999).

The analysis of fixation (F_{ST}) is the ratio of the total genetic variance found in a subpopulation (Meirmans & Hedrick, 2011). The F_{ST} value ranged from 0 to 0.05 are an indicator of lower genetic differentiation, F_{ST} ranged from 0.05 to 0.15 are an indicator of medium genetic differentiation, F_{ST} ranged from 0.15 to 0.25 is an indicator of higher genetic differentiation, and F_{ST} over 0.25 are an indicator of extreme genetic differentiation of breeds (Hartl & Clark, 1997; Hartl et al., 1980; Wright, 1978). Previously a lot of studies estimate F_{ST} estimated of different breeds; Rwandan chickens (0.054) (Habimana et al., 2020), Ethiopian chickens (0.048) (Dana et al., 2010), Kenyan chickens (0.003-0.040) (Mwacharo et al., 2007), Cameroonian chickens (0.08) (Keambou et al., 2014), Spanish chickens (0.244) (Davila et al., 2009), and Egyptian chickens (0.07) (Eltanany et al., 2011).

Additionally, the F_{ST} estimate of some breed was also estimated which was genotyped using high though put genotyping techniques; 7 Italian breeds (0.167) studied by NGS (Viale et al., 2017), 15 Chinese breeds (ranged from 0.03 to 0.27) studied by GWAS (Zhang et al., 2017), and 3 Chinese breeds (ranged from 0.0046 to 0.1530) studied by Illumina HiSeq 4000 sequencer (Zhang et al., 2018), 6 Italian breeds (0.437) (Zanetti et al., 2010) and Japanese breeds (0.429) (Tadano et al., 2008).

2.2. Genetics of plumage color

There are different colors associated with plumage color (Doucet et al., 2004; Johnson et al., 2012). However, the level of understanding of the molecular basis of the production process is poor. Color diversity is the result of two interrelated physical processes; optical and chemical factors (Makarova et al., 2019). The color of the plumage depends on gender, age, and shape of the cover (Rzepka et al., 2016). The main types of coloration in the feathers are melanin, porphyrin, and carotenoids (Lucas & Stettenheim, 1972). Melanin is a commonly available pigment in chicken (Yu et al., 2004). Carotene is absent in domestic chicks and adult feathers (McGraw et al., 2004), and it is also absent in Red Junglefowl adults (Thomas et al., 2014).

In birds, melanin is present in two forms: pheomelanin and eumelanin. Melanin is made from tyrosine and the aromatic amino acid (Makarova et al., 2019). Melanin is influenced by sex hormones (androgen and estrogen), thyroid hormone, and luteinizing hormone (Rzepka et al., 2016). It can also interact with other pigments and then give complex expressions of feather color. A dark brown allele is particularly interesting because it affects the nature of pigmentation, instead of the presence or absence of color (Zhang et al., 2017).

Melanogenesis is the process of color production (Kerje, 2003). During melanogenesis, many loci are involved, to makes the feathers more complex. Melanogenesis is primarily affected by genetics, but also by environmental and physiological conditions, and the production of the color varies depending on the sex, season of birth, and feather structure (Rzepka et al., 2016). The color of hair, skin, fur, and feathers in chickens and mammals is produced by melanocytes (Kerje, 2003).

As one of the major visual characteristics of the animals, color variations are used for breed identification and characterization (Fan et al., 2014). There are many factors involved in expressing the color of a plumage (Kerje, 2003). Genotype by environmental interactions (G*E) is the most contributor to the development of tumors and melanoma (Gudbjartsson et al., 2008; Ibarrola-Vilava et al., 2012). The color change in feathers is mainly due to changes in the levels of eumelanin and pheomelanin (Guernsey et al., 2013). There may be genes that control color diversity and have a pleiotropic effect and affect economic traits (Makarova et al., 2019).

The melanocortin 1 receptor (MC1R gene) is a protein that regulates hair color and skin color in mammals (Wolf Horrell et al., 2016). In poultry, MC1R is encoded by the solid black color locus (Kerje, 2003). The MC1R is encoded by a small gene (<1000 BP) and implanted in the melanocyte's membrane (Mundi, 2005). Black color is often the result of high levels of MC1R activity, and low levels contribute to pheomelanin production (García-Borrón et al., 2006). Mutations in the MC1R gene determine the zonal distribution of black eumelanin in the body of chickens and alter receptor activation (Hoque et al., 2013; Kerje, 2003).

In the past, a lot of studies have examined the genes involved in plumage color variation. According to Dávila et al. (2014), the change in the complementary sex determiner (CSD) locus E is explained by the haplotype of the MC1R gene. In quail, a lavender feather is associated with a variety of mutations in the melanophilin (MLPH) gene, resulting in weight loss in birds (Bedhom et al., 2012). Gunnarsson et al. (2011) indicated the location of the SOX10 gene on Chr 1 with more than 14 kb of dark brown color with a deletion of 8.3 kb.

Previously, a lot of research has been done on the secondary feather color technique. Adequate sources of information have been obtained from mice for the study of feather color, and about 150 loci associated with feather color have been found (Jackson, 1994). A mutation on the EDNRB2 gene affects the formation of marble feathers (Makarova et al., 2019). The synthesis of black melanin is activated by the enzymes TRP1 and TRP2 (Galvan et al., 2016). High levels of these enzymes are associated with darker colors in most birds, such as ducks, quail, geese, Chinese, and pigeons, and chickens.

Plumage color differences are the result of a variety of factors. The main determinant for plumage color differences in chickens is the amount of pheomelanin and eumelanin (Yang et al., 2017). Pheomelanin and melanin are also major constituents of brown, dark-black, and gray color. The formation of melanin cells is controlled by one major gene, AKT3 (Tsao et al., 2012). The AKT3 gene also regulates cell signaling, which is involved in growth factors, insulin, and various biological processes in the body.

Recently, a lot of significant SNPs and genes related to plumage color are identified. Yang and others. (2017) a study using Affymetrix 600K HD chip identified 13 significantly related SNPs with plumage color. Candidate genes NUA and SHH, and the synthesis of eumelanin are affected by these identified SNPs. Fogelholm et al. (2020) Identified five candidate genes significantly associated with red-brown color (ARL8A, CREBBP, LAD1, WDR24, and PHLDA3). Three different QTLs were also identified in Chr 2 at 149 cM, Chr 10 at 176 cM, and Chr 14 at 207cM.

Various studies have assessed the gene related to plumage color. A GWAS on Korean chickens identified 12 significantly related SNPs with plumage color (Park et al., 2013). According to Huang et al. (2020), 10-20% of the genes related to the color of feathers are located in Chr 2. A linkage mapping study on plumage color reported that Chr 1 and Chr 20 were significantly associated with plumage color (Kerje, 2003). According to Park et al. (2013), GWAS identified 8 SNPs most closely related to wing color in Korean chickens.

Unlike other animals, sex chromosomes in chickens are different, ZW and ZZ for hen and rooster respectively (Park et al., 2013). Sex-related locus “silver locus” controls silver, wild/gold color, and has interfered with red coloration (Gunnarsson et al., 2007). This result is an indicator of the relative color difference between hen and rooster. The colorful feathers in the roosters are related to the effect of the male chromosome when it comes to sex (Browner et al., 2000). The striped plumage color is influenced by sex-related and autosomal genes (Zhang et al., 2017).

Domestic animal colors are different from their wild ancestors. In the case of chickens, red junglefowl had a variety of feathers, varied from dark red to light orange. In commercial chickens, especially in broiler and layers, the plumage color is often only brown or white (Fogelholm et al., 2020). Various GWAS in livestock had conducted for coat color; such as cattle (Edea et al., 2017; Fan et al., 2014; Mastrangelo et al., 2019), goat (Chang et al., 2006; Nazari-Ghadikolaei et al., 2018), and sheep (Kijas et al., 2013; Muniz et al., 2016).

3. MATERIAL AND METHODS

3.1. Data source

All of the data used in the study were obtained from the Department of Animal Breeding and Genetics through my supervisor (Dr. Anna Maria Johansson), and some of the supplementary information was obtained from previous literature done on Swedish local chicken breeds. Chicken sample information such as the number of breeds used in the study, the number of individuals per breed, and the owner information was obtained from Dr. Anna Maria Johansson.

The number of individuals used for blood sample collection, the routine of blood sample collection, DNA extraction, DNA purity assessment, and the genotyping method information was obtained from Abebe and others (2015). The genetic marker information was obtained from Johansson and Nelson (2015). The genotyped data and phenotypic data including the photos of chickens were obtained from Dr. Anna Maria Johansson. The photos of the chicken were taken by Dr. Thomas Englund.

3.2. Chicken Samples

In this study, a total of 83 samples from eight breeds were used. The name of the breed and the sample size used in each breed were; Gotlandshöna chicken abbreviated to Gotl (N=24), Öländsk dvärghöna chicken abbreviated to ÖLDvärg (N=8), Kindahöna chicken abbreviated to Kinda (N=2), Hedemorahöna chicken abbreviated to Hedem (N=22), Skånsk blommehöna chicken abbreviated to SkBlom (N=8), Äsbohöna chicken abbreviated to Äsbo (N=3), Ölandhöna chicken abbreviated to Öland (N=4), and Svarthöna chicken abbreviated to Svart (N=12).

The chickens were obtained from 8 private owners of local chickens. 38 samples were collected from animals that were owned by an agricultural school, and the rest of the animals was owned by a member of the Swedish Local Chicken Association (Abebe et al., 2015). Chicken belonging to Äsbo, Kinda, and Öland were obtained from only one private owner. Svart, Gotl, and Hedem breeds were obtained from three owners. ÖLDVärg and SkBlom breeds were obtained from two owners. All of the breeds found in each owner were kept separately.

3.3. DNA-Samples and Genotyping

A total of 127 local chickens was used to collect the blood sample (Abebe et al., 2015). Of the total samples, in this study, 83 chickens were used, which was genotyped using an SNP chip. In all individuals, blood samples were taken using a small needle from the wing vein and the sample was mixed with EDTA at Eppendorf tube. The blood samples were collected by a local veterinarian. Before collecting blood samples, the ethical permission (permission number C247 / 6) was taken into account, obtained from the Uppsala Ethics Board (Swedish name: Uppsala djurförsöksetiska nämnd) (Johansson & Nelson, 2015).

Genomic DNA was extracted for all samples (Abebe et al., 2015). DNA purity was assessed by looking at the A260 / A230 and A260 / A280 ratios obtained by ultraviolet-visible spectroscopy. DNA purity was quantified and evaluated using the NanoDrop™ 8000 Spectrometer. Genotyping was performed in 2013 in Canada by DNA Landmark, and all of the marker's positions were based on the *Gallus_gallus*-2.1 genome assembly set from the SNP chip (Johansson & Nelson, 2015). 62K SNP chip was used for genotyping, created by Illumina for GWAS (Groenen et al., 2011).

3.4. Phenotypic traits used in the study

For the study of plumage color variability for the entire-body color and at different body parts a minimum number of three individuals per color group was considered. For the study of plumage color for the entire body; white, black, and black-white blooming plumage color was considered. For the study of plumage color of different body parts; wing, neck, and tail colors were considered. Only Gotl breed was also used to study plumage color variability, without considering the minimum number of individuals, for light-brown plumage color and black-tail color. There was an interest to use only Gotl breed to do GWAS, due to its relatively higher sample size to control population stratification. The sample of plumage colors of the chickens is shown in (Figure 1).



Figure 1. Svart chicken with black plumage color (Left); SkBlom chicken with black-white blooming plumage color (Right)

3.5. SNP quality control for diversity analysis

SNPs data were filtered using PLINK 1.9 programs. For the genetic diversity study, the data were analyzed by excluding individuals below the threshold. According to Purcell. (2007) Individuals with a genotyping rate >90% (i.e, less than 10% of missing information, mind 0.1), SNP >95% data (i.e, less than 5% of missing data, --geno 0.05), SNP >99% MAF (--maf 0.01), and SNP above the HWE test with $p < 10^{-9}$ were used for further analysis. Based on the above QC criteria, some SNPs have been removed as described in (Table 1). All chickens passed all the QC criteria, and there are no removed SNPs due to the HWE test. QC for all breeds was done at a genotyping rate of 95 %.

Table 1. SNP quality control

Breeds	Removed SNPs (geno)	Removed SNPs (MAF)	Passed SNPs after QC
Äsbo	2929	26726	23658
Gotl	3207	11206	38900
Hedem	3218	16508	33587
Kinda	3033	37644	12636
SkBlom	3555	10032	39726
Öland	3270	27666	22377
ÖLDvärg	3239	34278	15796
Svart	3542	26850	22921

Breeds: a breed used for quality control; Removed SNPs (geno): The number of SNPs removed due to missing genotype data at $geno=0.05$; Removed SNPs (MAF): the number of SNPs removed due to minor allele threshold(s) at $maf=0.01$; Passed SNPs after QC, the number of SNPs passed the quality control out of 53,313 markers for further analysis.

3.6. Statistical analysis

3.6.1. Diversity analysis

The basic dimensions of genetic diversity were calculated using the PLINK 1.9 program (Purcell et al., 2007). Heterozygosity levels are a reflection of genetic diversity within races (De et al., 2017). The observed homozygosity observed heterozygosity, and inbreeding coefficient (F) was computed using the --het function of the PLINK 1.9 program (Purcell, 2009). The observed heterozygosity was computed from the difference of non-missing count to the observed homozygous genotype count.

$$O(HET) = N(NM) - O(HOM)$$

Where O(HET) is the observed heterozygous count, which is the number of observed heterozygote genotypes found in an individual; O(HOM) is the observed homozygote genotype count, which is the number of homozygote genotypes found in an individual. N(NM) is the number of non-missing counts, which is the total

number of markers (the sum of observed homozygote and observed heterozygote genotype counts) found in an individual after quality control out of 53,313 markers. The inbreeding coefficient (F) was computed from the observed homozygote genotype count O(HOM) and expected homozygote genotype counts (E(HOM)), where E(HOM) is the expected number of homozygote genotypes found an individual under HWE.

$$F = ((O(HOM) - E(HOM)) / (N(NM) - E(HOM)))$$

The inbreeding coefficient (F) computed by PLINK corresponds to Wright's F-statistics estimate of FIS, where FIS is the global inbreeding of individuals within a breed. Wright's FIS has been initiated to quantify the excess of homozygosity or the deficiency of heterozygosity (Zhivotovsky, 2015). FIS (inbreeding) is the proportion of variance in an individual relative to the subpopulation. Where higher FIS is an indicator of a considerable level of inbreeding. Wright's FIS estimate has been computed by the following formula

$$\text{Wright's FIS} = E(HET) - O(HET) / E(HET)$$

Where E(HET) is an individual's expected heterozygosity under HWE, and O(HET) is an individual's observed heterozygosity. If $E(HET) > O(HET)$ then $FIS > 0$, indicating that an individual is inbred or there is inbreeding at this individual, while if $O(HET) > E(HET)$ then $FIS < 0$, indicating that an individual has more heterozygous genotype count than expected in HWE, as a result, there is no inbreeding at this individual.

Both estimates of inbreeding (F & FIS) are the same, and they are dependent on an individual's level of homozygosity and heterozygosity. If the O(HOM) is higher than the E(HOM) under HWE, then F will be positive which indicates a considerable degree of inbreeding in an individual. While if the O(HOM) is lower than the E(HOM) then F will be negative, indicating as this individual is not inbred as a result of a higher level of heterozygosity. If an individual had higher O(HOM) than the E(HOM), which is an indicator of a higher level of O(HET) than the E(HET) under HWE because the sum of O(HOM) and O(HET) give up one.

To interpret the estimated level of homozygosity and heterozygosity of the breeds more accurately the effective population size (Ne) of the breed was used. Since Ne is more useful to interpret the estimated population genetics parameters than the sample size of the breeds. Ne is the number of individuals that an idealized population needs to have, to have the same rate of inbreeding as the actual population (Gutiérrez et al., 2008). It has been computed by the formula below, where Nf is the number of female chickens found in the population, Nm is the number of male chickens found in the population of the breed.

$$Ne = \frac{4 * Nf * Nm}{Nf + Nm}$$

Minor allele frequency (MAF) is the frequency of rare alleles in a specific population and is used to measure heredity (Hernandez et al., 2019). SNPs with a $MAF \geq 0.05$ (5%) were used for the haplotype maps of the human genome to identify common genetic variation in humans (Belmont et al., 2005). MAF is also commonly used in the study of population genetics because it provides information on rare and common variants found in the population. In the current study, MAF is calculated by the --freq command of the PLINK 1.9 program (Purcell, 2009).

HWE testing is fundamental to identify genotyping errors in genetic markers (Weir & Graffelman, 2016). Departure from HWE is mainly due to genotyping error, but also due to genetic factors (Deng et al., 2001; Weir et al., 2004). The genetic factors that cause departure can be inbreeding, population admixture, heterozygous advantage, selection, and copy number variation. HWE principles have many assumptions; (1) random mating, (2) no selection, (3) no migration, (4) no mutations, and (5) large populations (Lachance, 2016). Violation of these assumptions will result in deviations from HWE. The deviation of markers from HWE is calculated using the --hardy command of the PLINK 1.9 program (Purcell, 2009).

PIC is important to measure the informativity and the usefulness of markers for linkage analysis (Guo & Elston, 1999). If the genetic markers are highly informative the estimated result of the studies will be more accurate for the inference of an individual's ancestry, while less informative markers are less sufficient to make an inference. PIC is calculated from MAF based on Botstein (1980). According to Botstein (1980) PIC estimated from p and q allelic frequencies, the probability of an individual being heterozygous is then $2pq$. Since we have biallelic markers (SNPs), so one of the allelic frequencies let say p, would be replaced by maf, then q would be simply $1 - maf$. Before calculation of PIC the heterozygosity has been calculated from $1 - (maf^2 + (1 - maf)^2)$ then it was subtracted for identical heterozygous trios $(2maf^2(1 - maf)^2)$.

$$PIC = 1 - (maf^2 + (1 - maf)^2) - (2maf^2(1 - maf)^2)$$

The pairwise genetic diversity of the breeds was estimated using F_{ST} . F_{ST} is the percentage of total genetic variation found in the subpopulation compared to the total genetic variant (Meirmans & Hedrick, 2011). The value of F_{ST} serves as a measure of genetic variation between populations (Takezaki and Nei, 2008). It is also correlated with the genetic distance of different groups. High F_{ST} is an indicator of significant genetic variation among populations. The F_{ST} value was analyzed using the --fst function of the PLINK 1.9 programs, by comparing the single markers between each of the paired breeds (Purcell, 2009).

3.6.2. Genetic relationship

The genetic relationship of the study breeds was computed using multi-dimensional scaling (MDS) plot. MDS plot is a method of displaying an individual's genetic similarity in a given set of data (Ahram et al., 2014). The MDS plot aims to set each individual in N-dimensional space; so that, the distance between individuals is

presented. MDS plot measures the population stratification using the “MDS function” of the R program. The input files for the R program were prepared in the PLINK 1.9 program using the --cluster and --MDS plot 2 commands (Purcell et al., 2007).

3.7. Genome-wide association test

62K SNP Illumina chicken array containing 53,313 SNPs distributed on the chicken genome were used for GWAS. GWAS was performed for phenotypic variability of different traits; plumage color variation for the entire body and different body parts. For the entire-body traits, GWAS was done to identify significantly associated SNP with white, black, and black-white blooming plumage color. For the identification of significantly associated SNPs with white plumage color Hedem breed was used; for black plumage color Gotl, Öland, Svart, and Hedem breeds were used; and for black-white blooming plumage color, SkBlom and Gotl breeds were used.

Additionally, GWAS was also done to identify significantly associated SNP with the color of different body parts; wing, neck, and tail colors. For the identification of significantly associated SNPs with black-wing color Gotl, Öland, and Äsbo breeds were used; for brown-black neck color Gotl, Äsbo, and Kinda breeds were used; and for black-tail color Gotl, Öland, Äsbo, Kinda, and Svart breeds were used. Quality control for GWAS analysis was performed by removing SNPs below the threshold. Individuals with a genotyping rate >75% (i.e., less than 25% of missing information, mind 0.25), SNP >75% data (i.e., less than 25% of missing data, --geno 0.25), and SNP >95% MAF (--maf 0.05) were used for GWAS (Purcell, 2007). After QC, GWAS was performed with --assoc and --adjust command of the PLINK 1.9 program (Purcell, 2007). Then the result was plotted with the R package “qqman” (Park et al., 2013).

As a standard indicator of GWAS results; P-value is represented in two ways; the Manhattan plot and QQ plot (Purcell, 2007). The p-value of each SNP is based on their base pair position along each of the autosomal chromosomes. The x-axis in the Manhattan plot represents the P-value based on chromosome sequence and position on the chromosome, and the y-axis represents each of the genome-wide SNPs ($-\log_{10}$ (P-values)). The QQ plot represents the observed p-value relative to the expected P-value of markers, where, the x-axis represents the expected p-value at ($-\log_{10}$ (P-values)) and the y-axis represents the observed p-value at ($-\log_{10}$ (P-values)).

As a result of too high genomic inflation factor (lambda) in the GWAS computed from the --assoc and --adjust command done previously; principal components (PCs) were used as covariates in the GWAS to control the effect of population stratification. The first three PCs of the Gotl breed were used as covariates in the GWAS analyses to control population stratification between subpopulations. Gotl breed has been used because of its relatively high sample size, to control population stratification.

According to Chang (2020) Incorporating PCs as covariates help to control the confounding effect of population stratification. A bi-plot PCs was plotted after QC; by removing SNPs >10% missing data (geno 0.1), removing individual's >10% missing information (mind 0.1), and removing SNPs < 95% MAF (maf 0.05) (Chang, 2020). The GWAS was performed using logistic regression (--logistic command) in the PLINK 1.9 program. In this study, all of the analyses were based on the autosomal markers (Chr 1-28) using Gallus_gallus-2.1 genome assembly (Johansson & Nelson, 2015).

4. RESULT and DISCUSSION

4.1. Genetic diversity within and between breeds

The basic measures of genetic diversity are summarized in (Table 2). The estimated homozygosity and heterozygosity of the breeds were interpreted concerning their effective population size (N_e) and sample size. Before calculating the N_e of the breeds, the population size of the breeds was obtained from (http://kackel.se/lantras_rodlista.html). N_e is the number of individuals that an idealized population needs to have, to have the same rate of inbreeding as the actual population (Gutiérrez et al., 2008).

The N_e of the breeds was highly varied; ÖLDVärg had N_e of 251, Svart had 259, Öland had 423, Gotl had 543, SkBlom had 668, Kinda had 762, Äsbo had 1188, and Hedem breed had N_e of 1179. Most of the breeds with a relatively lower N_e had lower observed homozygosity than expected. On the other side, a breed with higher N_e had higher observed homozygosity than the expected (Hedem breed). Following Hedem breed, Äsbo and Kinda breed had a relatively higher N_e and showed lower observed homozygosity than expected.

Gotl and SkBlom breed had N_e of 543 and 668 respectively, which is relatively lower than N_e of Äsbo and Kinda breed showed higher observed homozygosity than expected. Theoretically, a breed with a smaller N_e will have lower diversity, while a breed with a larger N_e will have a higher diversity. Some of the breeds with a larger N_e had also lower observed homozygosity than expected, while some of the breeds with smaller N_e had lower observed homozygosity than expected. The estimated level of homozygosity and heterozygosity in some of the breeds is highly varied from the theoretically expected level of diversity based on their N_e . This deviation may have happened as a result of the sample size, which may not be a true representative of the population size of the breeds.

The estimated average observed homozygosity of the breeds varied from 0.39 to 0.73 in the Kinda and Hedem breed respectively. The average expected homozygosity varied from 0.58 in the Kinda breed to 0.68 in Hedem and Gotl breed. Lower observed homozygosity found at Kinda, Äsbo, Öland, ÖLDVärg, and Svart breed than their expected level of homozygosity under HWE. Those breeds showed on the other side higher heterozygosity level, because if a breed had lower observed homozygosity than the expected under HWE, it is an implication of excess of heterozygosity within that breed.

The lower level of observed homozygosity (higher observed heterozygosity) within a breed may have been associated with the isolation break effect. The isolation break effect is a phenomenon when the heterozygosity temporarily increased as a result of the interbreeding of discrete subpopulations, which results in the decrease of homozygosity (Borowsky, 1987). The mixing of previously isolated breeds results from an increase of observed heterozygosity than the expected heterozygosity. The isolation-break effect in our study breeds occurred as a result of the mixing of two isolated populations because owners of the same breed had exchanged their birds. Therefore, an increase of heterozygosity in our study breeds has been associated with an isolation break effect.

Some breeds had higher observed homozygosity than the expected under HWE; SkBlom, Hedem, and Gotl breed had higher observed homozygosity than expected. A breed with a higher homozygous genotype had a lower heterozygous genotype since the sum of these genotypes give one. A breed having a higher number of homozygous genotypes is highly inbred and resulted to have a higher F. When a breed had higher observed homozygosity than expected the observed heterozygosity is lower (heterozygosity deficit), and it is an indicator of excess of homozygosity within that breed. There is a lower level of within-breed diversity in those breeds because the level of heterozygosity is an implication of genetic diversity. Heterozygosity levels are a reflection of genetic diversity within the breed (De et al., 2017).

Heterozygosity deficit within a breed may be due to the presence of homozygous alleles at the same locus (Dakin and Avis, 2004). A low level of within-breed diversity can be related to small N_e , inbreeding, genetic drift, null allele effect, and breeding practices, and lack of effective breeding strategies (Aubin-Horth et al., 2005; Dakin & Avise, 2004). Keeping breeds in small populations and small isolated herds for many generations also results in the loss of heterozygosity (Young & Clark, 2000). A breed having higher observed homozygosity than the expected had lower heterozygosity and a positive F. This is an indicator of mating among genetically related individuals. The observed homozygosity may also increase as a result of genetic drift and non-random mating.

The mean observed heterozygosity varied from 0.27 for Hedem to 0.61 for the Kinda breed. The average observed heterozygosity of the study breeds was 0.40, indicating that the Swedish chicken breeds had 40% genetic variability. The observed heterozygosity estimate for Swedish chicken breed (0.40) was relatively lower than; Turkish native chickens (0.665) (Kaya and Yildia, 2008), Zimbabwean native chickens (0.5) (Muchadeyi et al., 2007), Vietnamese native chickens (0.62) (Cuc et al., 2010), and Red Jungle Fowl (0.60) (Berthouly et al., 2009) using microsatellite loci.

Other studies, such as; for Chinese chickens (0.64) (Grnevitze et al., 2007), and five Swedish native chickens (0.612) (Abebe et al., 2015) studied using 20 microsatellite loci reported a larger estimate of heterozygosity than the current study result. Although, the heterozygosity estimate was relatively higher than that of Italian native chickens (0.35) (Zanetti et al., 2010). The level of heterozygosity in chickens appears to be low compared to other animals; for instance, fish (0.65, Rutten et al.,

2004), cattle (0.52, Sodhi et al., 2005), pig (0.74, Behl et al., 2006), and also humans (0.72, Ayub et al., 2003).

Five breeds were significantly deviated from Hardy Weinberg's expectation, at $p < 0.05$ (Table 2). Hedem breed had a higher number of markers significantly deviated from HWE, followed by SkBlom, Svart, Gotl, and ÖLDVärg breeds respectively. This finding is consistent with the findings of Abebe and others (2015) from a study of genetic diversity on those five breeds. This consistency is observed at the same samples in each breed in both studies, except the genotyping techniques (the previous study samples genotyped using 24 microsatellite markers)

Departure from HWE is mainly due to genotyping error, but also due to genetic factors (Deng et al., 2001; Weir et al., 2004), inbreeding is one of the genetic factors causing the deviation of markers from HWE. The number of markers that deviated from HWE is in parallel to their level of F, higher F results in deviation of a high number of markers. For instance, the Hedem breed had the highest F (0.17) and had the highest number of markers deviated from HWE (4951 markers), and the other breeds follow this trend based on their level of inbreeding. Based on the current estimate of F, deviation from HWE may be related to inbreeding within breeds.

Inbreeding (F) is the excess of homozygosity than expected under HWE. The estimated F in PLINK is equivalent to the FIS estimate of Wright's statistics, and they depend on an individual's level of homozygosity and heterozygosity. FIS is the global inbreeding of individuals within a breed, which is computed from an individual's heterozygosity and homozygosity as F estimate of PLINK. As shown in (Table 2), the F estimate in most of the breeds is negative. In the sense of FIS, a negative estimate is an indicator of higher heterozygosity of individuals than expected, which is the same interpretation of F. The average F of the study breeds calculated from the discrepancy of observed and expected heterozygotes was -0.07, indicating most of the Swedish chicken breeds were not inbred.

The inbreeding coefficient (F) in Gotl breed individuals was varied from -0.45 to 0.41, where 8 chickens had negative F, 2 chickens had zero F and others had a positive F, the average F of the breed has been 0.06. There is higher variability in F of individuals. The estimated F in Hedem breed chickens was varied from -0.06 to 0.45, where 2 chickens had negative F and others had positive F, and the average F of the breed has been 0.17. There is also a higher level of variability in F of individuals. The estimated F in individual chickens of the Kinda breed was -0.46 and -0.48, where the average estimated F of the breed was -0.47. The estimated F of Äsbo breed chickens was -0.30, -0.37, and 0.35, and the average F of the breed was -0.10.

The estimated F of Öland breed chickens was -0.27, -0.13, 0.05, and 0.26, where the average F has been -0.02. The estimated F of the ÖLDVärg breed chickens was varied from -0.50 to 0.10, all of the breeds have negative F except one chicken (0.10). The average estimated F of the breed was -0.23. The estimated F of SkBlom breed chickens was varied from -0.23 to 0.30, only two chickens had negative F, and the average F of the breed has been 0.13. The estimated F of Svart breed

chickens was in the range of -0.44 to 0.20, 7 chickens had negative F, and the average estimate of F of the breed was -0.09.

The average inbreeding coefficient (F) of the breeds was ranged from -0.47 to 0.17 (Table 2). The highest F was observed at Hedem breed (0.17), while the lowest F was observed at Kinda breed (-0.47). The estimated F of Svart (-0.09) and Hedem (0.17) breed was the same as with the finding of Johansson and Nelson (2015) from the diversity study on those two breeds. This estimated F of Svart and Hedem breed was conducted from the same individuals with equal sample size in both works, rather than the genotyping techniques (the previous study samples were genotyped using 60K SNP chip). The estimated level of inbreeding is dependent on the level of homozygosity and heterozygosity.

A negative estimated F indicates that the chickens are more heterozygous than expected in HWE. For instance, the Kinda breed had the highest heterozygosity (0.64) and the lowest homozygosity (0.39), as a result, it had the lowest F (-0.47), while the Hedem breed had the lowest heterozygosity (0.27) and the highest homozygosity (0.73), as a result, showed the highest F (0.17) (Table 2). Hedem, Gotl, and SkBlom breed had a positive F; Hedem (0.17), Gotl (0.06), and SkBlom (0.13) as a result of a higher level of observed homozygosity than expected homozygosity. Inbreeding within a breed may be a result of non-random mating and genetic drift (Nichols, 2017).

The polymorphic information content (PIC) is important to measure the informativity and usefulness of markers for linkage analysis (Guo & Elston, 1999). It is also the best indicator for the estimation of the polymorphism of marker locus. PIC is a preferable way to measure how much a certain marker has a contribution to the inference on a certain ancestry. Highly informative markers reduced the required amount of genotyping and the number of markers for genetic predisposition (Rosenberg et al., 2003). PIC for the co-dominant markers is ranged from 0 (monophonic) to 1 (very informative, with many alleles having equal frequencies) (Smith et al., 1997). Therefore, polymorphic alleles are preferable to monomorphic ones.

The average PIC of the study breeds was 0.25, the highest PIC observed in Kinda and Svart (PIC=0.33), while the lowest was observed in Öland, ÖLDVärg, and Svart breeds (0.19) (Table 2). According to (Dakin & Avise, 2004) $PIC > 0.5$ is more informative, $0.25 < PIC < 0.5$ is moderately informative, and $PIC < 0.25$ is slightly informative. Therefore, a marker having a PIC higher than 0.5 is more recommended for genetic research, but lower than 0.25 is not recommended. The genetic markers of Kida (PIC=0.33), Svart (PIC=0.33), Äsbo (PIC=0.31), and Hedem breed (PIC=0.25) were moderately informative. Although, the genetic markers of Gotl, SkBlom, Öland, and ÖLDvärg had a $PIC < 0.25$, which is less informative.

Using genetic markers is important in a variety of contexts, including the admixture, gene flow, association mapping, and evolutionary history (Shriver et al., 1997, Bamshad et al., 2003; Ziv & Burchard, 2003). Therefore, if the genetic markers are highly informative such studies would be more accurate for the inference of an

individual's ancestry, while less informative markers are less sufficient to make an inference. Based on our finding the genetic markers of some of the breeds had a moderately informative marker (Kinda, Äsbo, Svart, and Hedem breed), and some of the breeds (Öland, ÖLDVärg, Gotl, and SkBlom breed) had slightly informative markers, and the average PIC of the overall population was moderately informative. Therefore, it would be preferable to use high-throughput genotyping techniques to our study breeds for the inference of an individual's ancestry more accurately, instead of the 62K SNP chip we have used.

The average PIC of the study breeds (0.25), indicating the moderate level of informativity of the genetic markers of the study breeds, which is lower than Egyptian chicken breed (PIC=0.61) (Eltanany et al., 2011), Italian breed (0.54) (Zanetti et al., 2010), southern Xinjiang chicken breed (0.79) (Azimu et al., 2018) and Swedish breed (0.56) (Abebe et al., 2015) studied using 24 microsatellite markers. The average MAF of the study breeds was 0.24, with the lowest value of 0.08 in Kinda and the highest value of 0.30 in Äsbo and ÖLDVärg breeds (Table 2). MAF is the frequency of rare alleles found in a population (Hernandez, 2019). Therefore, Äsbo and ÖLDVärg breed had moderately rare alleles, while the Kinda breed had a small number of rare alleles.

SNPs with a MAF \geq of 0.05 (5%) were used for the haplotype maps of the human genome to identify common genetic variation in humans (Belmont et al., 2005). MAF is also commonly used in the study of population genetics because it provides information on rare and common variants found in the population. The estimated average MAF of all of the study breeds was above 5 %, therefore plenty of information can be obtained from the Swedish chicken breeds for population genetics study.

Table 2: The measure of genetic diversity for the study breeds

Breed	Sample size	O(HOM)	E(HOM)	O(HET)	F	MAF	PIC	N d-HWE
Kinda	2	0.39	0.58	0.61	-0.47	0.08	0.33	NS
Äsbo	3	0.57	0.61	0.43	-0.10	0.30	0.31	NS
Öland	4	0.63	0.64	0.37	-0.02	0.27	0.19	NS
ÖLDVärg	8	0.53	0.62	0.47	-0.23	0.30	0.19	92
Svart	12	0.62	0.66	0.38	-0.09	0.26	0.33	641
SkBlom	8	0.69	0.65	0.31	0.13	0.27	0.19	1385
Hedem	22	0.73	0.68	0.27	0.17	0.24	0.25	4951
Gotl	24	0.70	0.68	0.30	0.06	0.19	0.20	288
Overall	83	0.60	0.64	0.40	-0.07	0.24	0.25	

Sample size, the number of individuals per breed; Overall, the average estimate of parameters from the breeds, MAF, the average minor allele frequency; PIC, the mean polymorphism information content; O(HOM), the mean observed homozygous genotype; E(HOM), the mean expected homozygous genotype; O(HET), the mean observed heterozygous genotype; F, the mean inbreeding coefficient estimate; HWE-departure (N d-HWE), the number of markers significantly deviated from HWE test at p-value <0.05; NS, no significance deviation from HWE at p-value <0.05

The average pairwise fixation index (FST) of the study breeds is shown in (Table 3). The average pairwise FST of the total population was 0.36, and the highest FST (0.5) was observed between ÖLDvärg and Kinda breed, and the lowest FST (0.14) was observed between the SkBlom and Äsbo breed. FST values of 0 to 0.05 show low genetic variation, FST value ranged from 0.05 to 0.15 show moderate genetic variation, FST value of 0.15 to 0.25 show significant genetic variation, and FST value above 0.25 show extreme genetic variation between breeds (Hartl & Clark, 1997; Hartl et al., 1980; Wright, 1978).

The average pairwise FST between most of the study breeds was extremely higher (FST >0.25) (Table 3), between Hedem and Äsbo, Hedem and Gotl, Kinda and Äsbo, Kinda and Hedem, Öland and Äsbo, Öland and Kinda, ÖLDVärg and Äsbo, ÖLDVärg and Gotl, ÖLDVärg and Hedem, ÖLDVärg and Kinda, ÖLDVärg and SkBlom, ÖLDVärg and Öland, was above 0.25. Additionally, the FST estimate between Svart and Äsbo, between Svart and Gotl, between Svart and Hedem, between Svart and Kinda, between Svart and SkBlom, between Svart and Öland, between Svart and ÖLDVärg was also above 0.25. It is an indicator of extreme genetic variation between most of the breeds.

The pairwise FST estimate between Kinda and Äsbo, between SkBlom and Gotl, between Öland and Gotl, between SkBlom and Kinda, and between Öland and SkBlom was higher (FST ranged from 0.15 to 0.25). This higher FST value between these breeds is an indicator of significant genetic differences between those paired breeds. The lowest pairwise FST (0.14) was observed between SkBlom and Äsbo breeds but indicates some level of genetic variation.

Table 3. Pairwise FST estimate between breeds

Breed	1	2	3	4	5	6	7	8
1 Äsbo								
2 Gotl	0.21							
3 Hedem	0.27	0.27						
4 Kinda	0.31	0.25	0.27					
5 SkBlom	0.14	0.18	0.25	0.16				
6 Öland	0.26	0.23	0.31	0.36	0.19			
7 ÖLDvärg	0.44	0.32	0.35	0.50	0.29	0.45		
8 Svart	0.39	0.32	0.35	0.42	0.29	0.40	0.44	
Overall=	0.36							

Pairwise diversity of breeds using wright's FST estimate, via Weir and Cockerham's method (1984); 1= Äsbo; 2=Gotl; 3=Hedem; 4=Kinda; 5=SkBlom; 6=Öland; 7=ÖLDvärg; 8=Svart; Overall, the mean FST estimate of the study population

The average FST value of the study population was 0.36, indicating an extreme genetic difference between Swedish chicken breeds. Compared to other studies, the FST value of the present study breeds was higher than; Rwandan chickens (0.054) (Habimana et al., 2020), Ethiopian chickens (0.048) (Dana et al., 2010), Kenyan chickens (0.003-0.040) (Mwacharo et al., 2007), Cameroonian chickens (0.08)

(Keambou et al., 2014), Spanish chickens (0.244) (Davila et al., 2009), and Egyptian chickens (0.07) (Eltanany et al., 2011).

The average F_{ST} of the study population (0.36) was also higher than that of other breeds studied by large scale SNP and sequence-based studies; 7 Italian breeds (0.167) studied by NGS (Viale et al., 2017), 15 Chinese breeds (ranged from 0.03 to 0.27) studied by GWAS (Zhang et al., 2017), and 3 Chinese breeds (ranged from 0.0046 to 0.1530) studied by Illumina HiSeq 4000 sequencer (Zhang et al., 2018). However, it was lower than that of 6 Italian breeds (0.437) (Zanetti et al., 2010) and Japanese breeds (0.429) (Tadano et al., 2008).

4.2. Genetic relationship between breeds

The multi-dimensional scaling (MDS) plot is a method of displaying an individual's genetic similarity based on their relative position in a given set of data (Ahram et al., 2014). MDS plot of the study population is shown in (Figure 2). In the MDS plot, the study breeds formed three main clusters based on their genetic relationship. In the MDS plot most of the study breeds were clustered together; Äsbo, Kinda, Öland, ÖLDvärg, SkBlom, and Gotl breeds were clustered together and forming the 1st clusters. It is an indicator of some degree of genetic relationship between these breeds. But, only 3 individuals of the Gotl breed were closer to other breeds, which may be corresponding to differences in the flock they were kept.

Svart and Hedem breed formed a cluster consist of 2nd and 3rd clusters separately from others. Therefore, Svart and Hedem breeds are distantly related to that of others. Relative to the Svart, the Hedem breed is very separated from others. A few breeds in the MDS plot didn't match the F_{ST} estimate of the pairwise diversity between breeds especially in cluster one, perhaps due to inbreeding of individuals. Some of the breeds which have a higher pairwise F_{ST} estimate were clustered together in the MDS plot. The F_{ST} value measures the allelic frequency and is dependent on the sample size, while the MDS plot is dependent on the genotype of individuals.

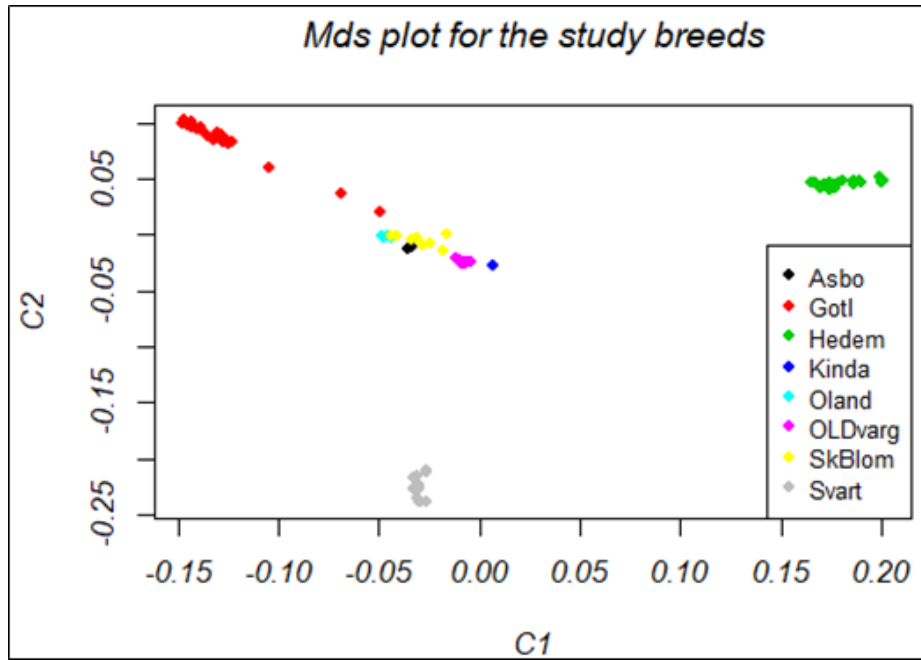


Figure 2. MDS plot of the study breeds; MDS plot indicated distance and similarity between breeds, C1 is the largest component of variability, and C2 indicated the second-largest component of variability

4.3. GWAS for phenotypic traits

GWAS was performed for phenotypic variability of different traits; plumage color variation for the entire body and different body parts. For the entire-body traits, GWAS was done to identify significantly associated SNP with white, black, and black-white blooming plumage color. Additionally, GWAS was also done to identify significantly associated SNP with the color of different body parts; wing, neck, and tail colors. There was a problem of population stratification in Swedish chicken breeds in all of the GWAS analyses, even within one breed.

Although the Manhattan plot showed significant SNPs associated with plumage color on the entire-body trait; white, black, and black-white blooming plumage color (Appendix 1) and in different body parts; wing, neck, and tail color (Appendix 2), the P-value in the QQ plot in all GWAS highly deviated from the theoretical P-value (Appendix 1&2). Deviation of the observed P-value from the theoretical P-values was higher in all GWAS analyses due to a higher genomic inflation factor ($\lambda > 1$). An example of the QQ plot is shown in (Figure 3), the remaining plots are found in the Appendix part.

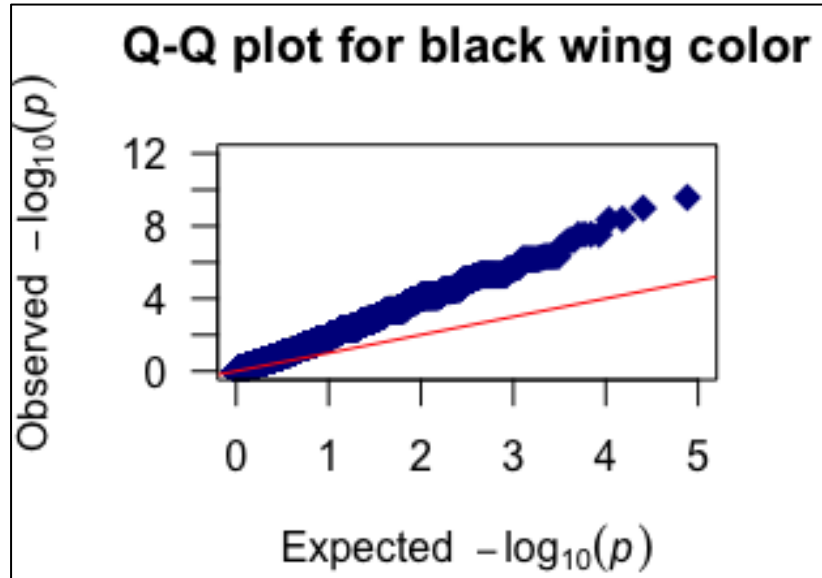


Figure 3. Q-Q plot for black wing color with inflation factor ($\lambda=2.4265$)

According to Clayton and others (2005) $\lambda=1$ indicates that there is no population stratification and $\lambda>1$ indicates the existence of population stratification. λ is computed from the ratio of the median of the association test to the expected median values under the null distribution (Devlin & Roeder, 1999; Reich & Goldstein, 2001). The λ value for all GWAS analyses was higher; for instance, white plumage color had $\lambda=2.41228$, black plumage color had $\lambda=2.70885$, and black-tail color had $\lambda=2.39234$.

Higher λ value in all analyses is an implication of the problem of population stratification in the Swedish chicken breeds. Thus, GWAS analyses generally failed to identify significant SNP's associated with plumage color (white, black, and black-white blooming plumage color) and different body parts (wing, tail, and neck color). Once λ has been identified from the above GWAS computed by -- assoc and --adjust command, a breed with a relatively higher sample size (Gotl breed) has been used to do GWAS by incorporating principal components (PCs) as covariates.

According to Chang (2020) incorporating PCs as covariates help to control the confounding effect of population stratification. Population stratification is an indication of systematic differences in the allelic frequency of the subpopulations (Prince et al., 2010). To control the effect of population stratification, Gotl breed (N=24) was used to do GWAS by incorporating PCs as covariates. A bi-plot PCs of the Gotl breed (Appendix 3), showed that individuals were split into two main subpopulations. Then, the first 3 PCs were used as covariates in the GWAS for light-brown plumage color and black-tail color.

After correction, there is no significant association instead the p-values had been extremely lower in the Manhattan plot, and the QQ plot also showed that all of the p-values have been too low as shown in (Figures 4&5). The use of PCs then seems to give too strict correction than the previous GWAS. It was also tried to see the structure of the breed using an MDS plot to check the homogeneity of the color of

individuals in the subpopulation, but there was no similarity of an individual's plumage color which was grouped in one cluster. The reason for the loss of homogeneity is related to a smaller number of individuals who have the same plumage color since we didn't consider the minimum number of individuals in this breed.

The population structure in Swedish chicken breeds is due to the large differences between flocks of the same breed from different owners. If we would have a larger sample size from the same owner the GWAS of only a single flock might have worked, but since most owners of these local breeds have small flocks, it is very difficult to find an owner with a large number of birds. Generally, GWAS failed to identify significantly associated SNPs with plumage color for the entire body part as well as different body parts.

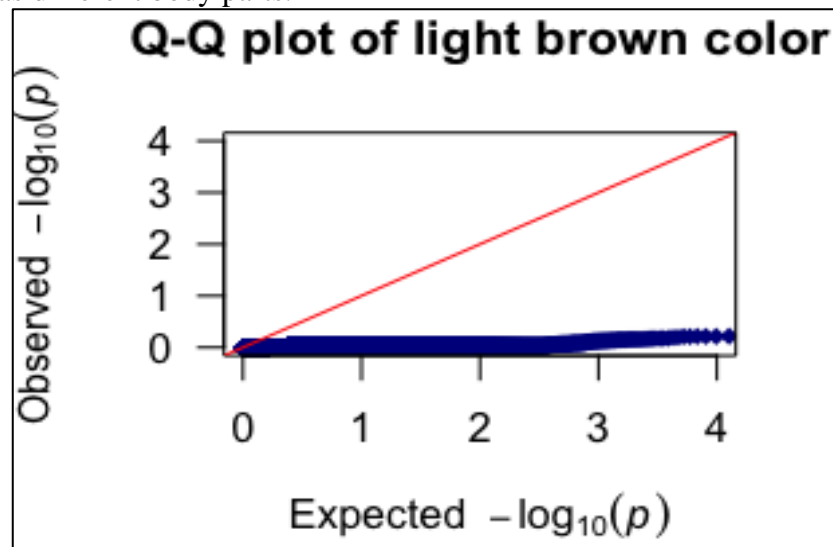


Figure 4. QQ plot for Gotlands breed for light-brown plumage color using principal components

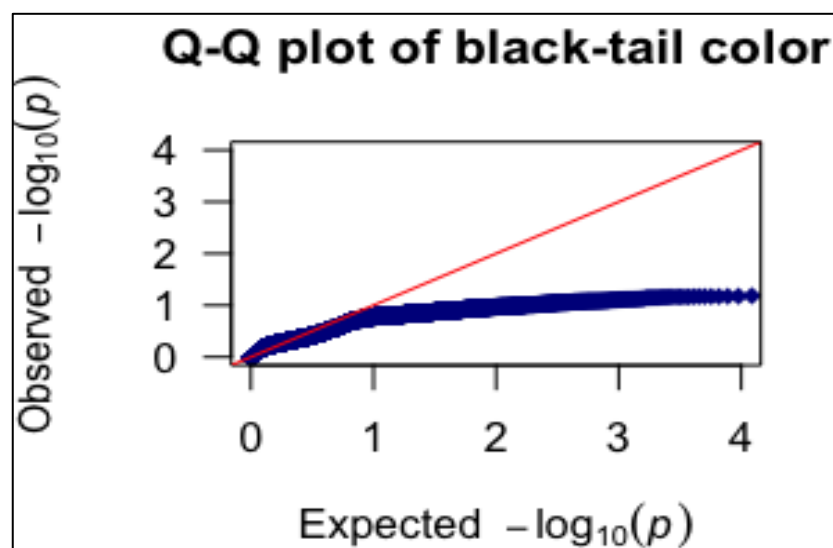


Figure 5. QQ plot for Gotlands breed for black-tail plumage color using principal components

5. CONCLUSION

Overall, Swedish chicken breeds were genetically diverse. The study breeds were grouped into three main groups based on their genetic relationship. Most of the breeds were grouped together in one of the main groups. Some levels of inbreeding were detected in our samples from Hedem, SkBlom, and Gotl breeds. Due to population structure, it was not possible to do GWAS in Swedish chicken breeds. The current study in genetic diversity may help to strengthen the genetic conservation program; for instance, to eliminate inbreeding and future genetic-based studies in Swedish chicken breeds.

It is very important to properly manage and protect the existing genetic variation in local Swedish chickens. Owners of the inbred chickens should get an animal that is with low relatedness to the current birds found in the flock. To do GWAS for phenotypic variability of Swedish chickens, the sample size must be much larger. Further research into phenotypic variability should be done by including many more individuals.

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Acknowledgments

First of all, I would like to thank the Almighty God for everything. I would also like to express my sincere gratitude to the Department of Animal Breeding and Genetics (SLU) for providing me all the necessary data for this thesis work. I would like to express my deepest gratitude to my supervisors; Dr. Anna Maria Johansson and Prof. Dr. Henner Simianer. I have a special thanks for European Masters in Animal breeding and genetics (EMABG), for allowing me to study at two giant European universities (Georg August University of Göttingen and Swedish University of Agriculture), including financial sponsorship.

I would like to express my gratitude to my family and friends for all of the love, support, encouragement, and prayers they have sent my way along this journey. I would like to express my most profound appreciation to my spouse Dr. Mulat Chanie for your love, patience, continuous encouragement, and support throughout my study. Heartfelt thanks and appreciation go to my friend Mr. Legese Abebaw, for your very generous support in all of my study period. Finally, I would like to say thank you to EMABG staff who have helped me through different aspects throughout my study period; Ms. Nathalie Schwaiger, Dr. Brianne Altmann, and Prof. Dr. Gábor Mészáros.

Appendix 1: GWAS for the entire body parts

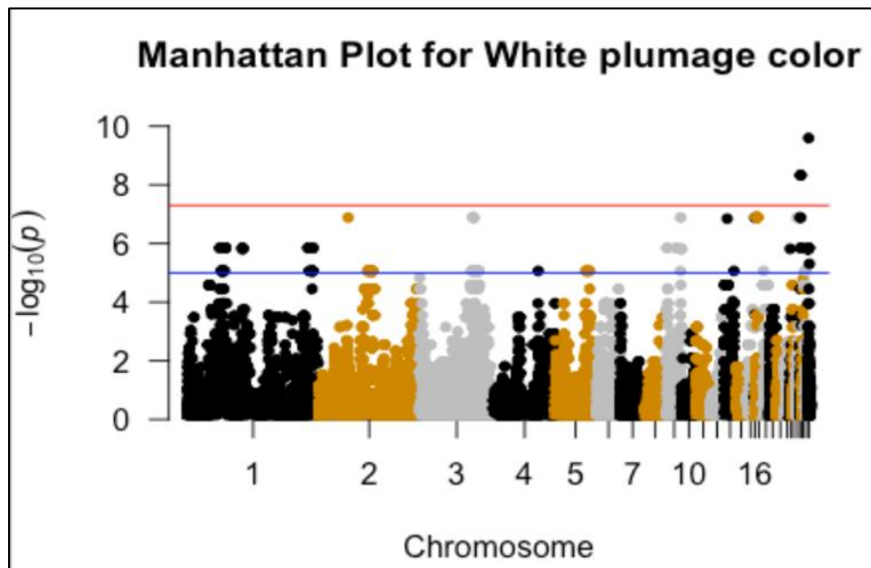


Figure 1. Manhattan plot for White plumage color

The y-axis indicates the $-\log_{10}(P\text{-values})$ of the genome-wide SNP in each GWAS meta-analysis. The blue horizontal lines represent suggestive lines ($-\log_{10}(1e-05)$, at $p < 0.005$) and red line represent the Bonferroni-corrected genome-wide significance (at $-\log_{10}(5e-08)$ at $p < 0.001$)

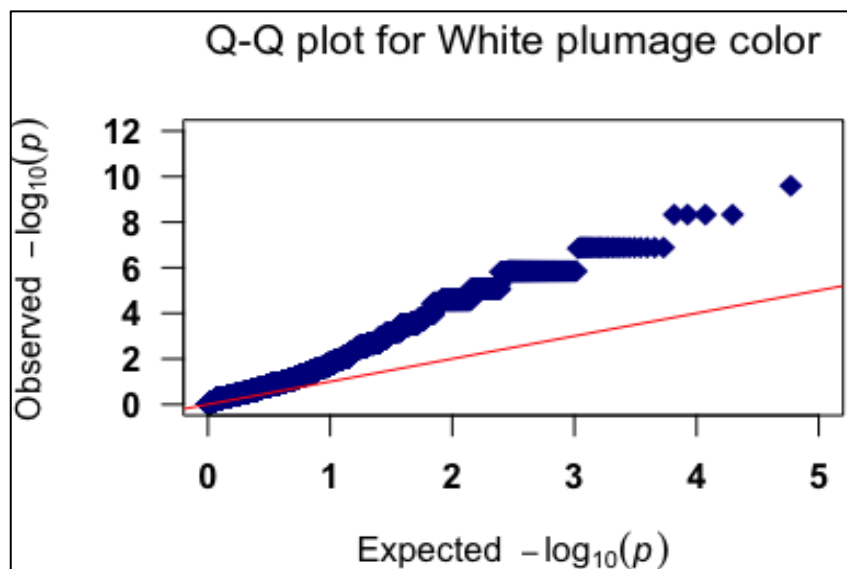


Figure 2. QQ plot for White plumage color with inflation factor ($\lambda=2.41228$)

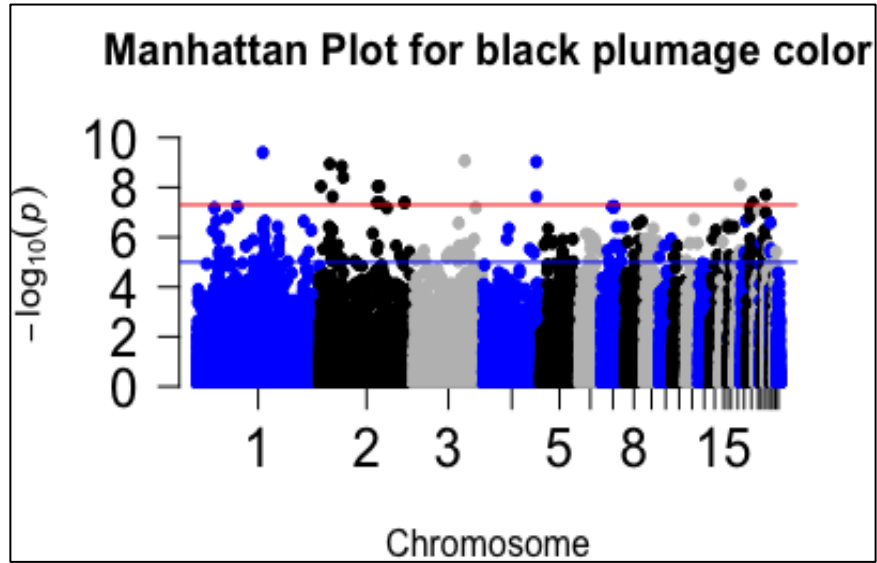


Figure 3. Manhattan plot for Black plumage color

The y-axis indicates the $-\log_{10}(P\text{-values})$ of the genome-wide SNP in each GWAS meta-analysis. The blue horizontal lines represent suggestive lines ($-\log_{10}(1e-05)$, at $p < 0.005$) and red line represent the Bonferroni-corrected genome-wide significance (at $-\log_{10}(5e-08)$ at $p < 0.001$)

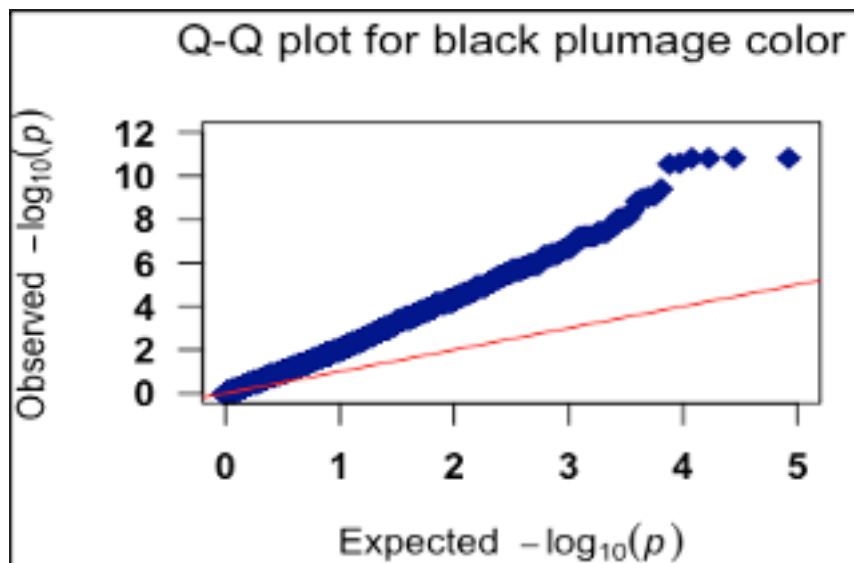


Figure 4. QQ plot for Black plumage color with inflation factor($\lambda=2.70855$)

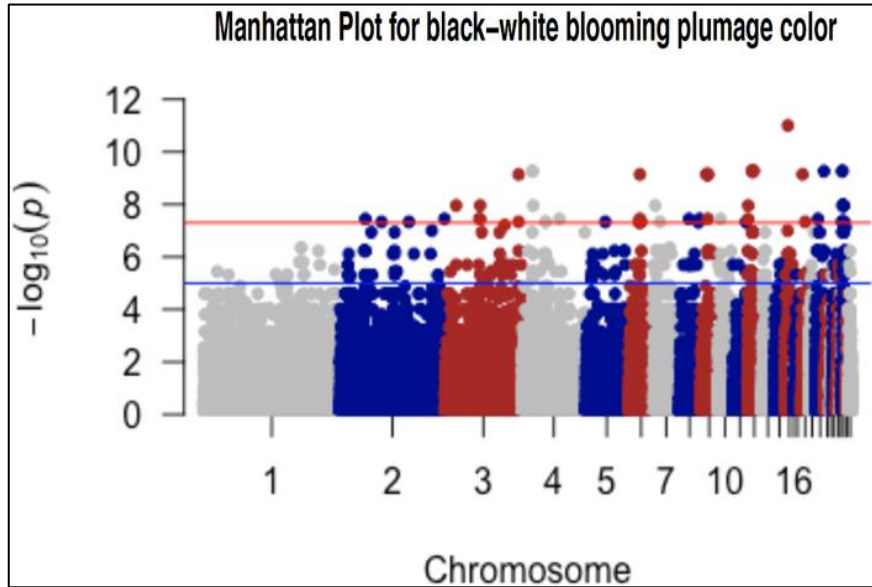


Figure 5. Manhattan plot for black-white blooming plumage color

The y-axis indicates the $-\log_{10}(P\text{-values})$ of the genome-wide SNP in each GWAS meta-analysis. The blue horizontal lines represent suggestive lines ($-\log_{10}(1e-05)$, at $p < 0.005$) and red line represent the Bonferroni-corrected genome-wide significance (at $-\log_{10}(5e-08)$ at $p < 0.001$)

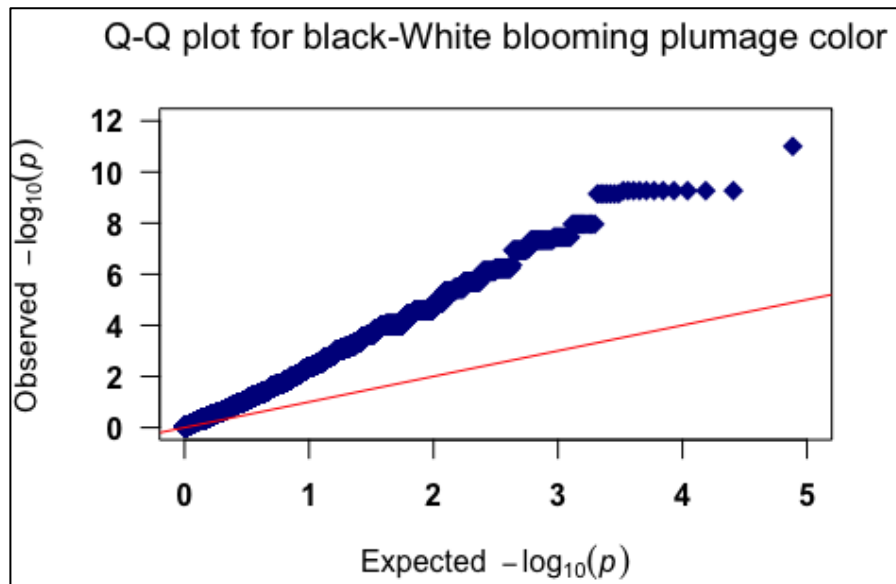


Figure 6. QQ plot for Black-white blooming plumage color with inflation factor ($\lambda=2.8643$)

Appendix 2: GWAS for different body parts

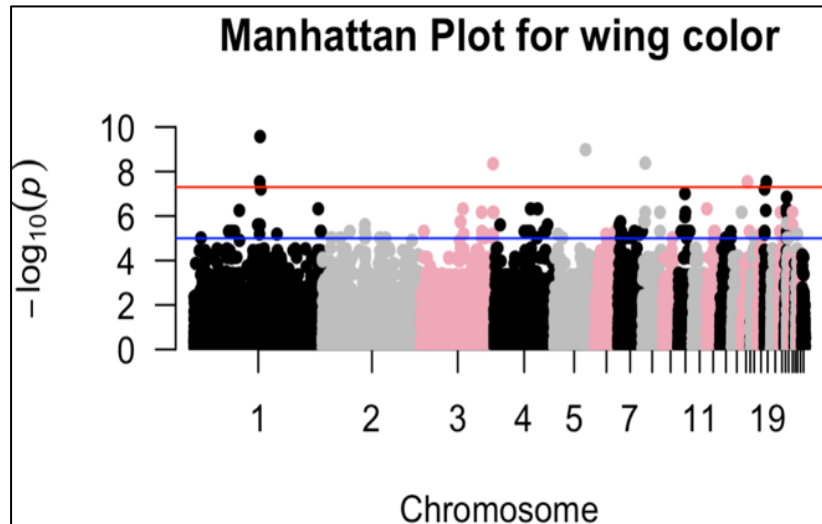


Figure 1. Manhattan plot for Black-wing color

The y-axis indicates the $-\log_{10}$ (P-values) of the genome-wide SNP in each GWAS meta-analysis. The blue horizontal lines represent suggestive lines ($-\log_{10}(1e-05)$, at $p < 0.005$) and red line represent the Bonferroni-corrected genome-wide significance (at $-\log_{10}(5e-08)$ at $p < 0.001$)

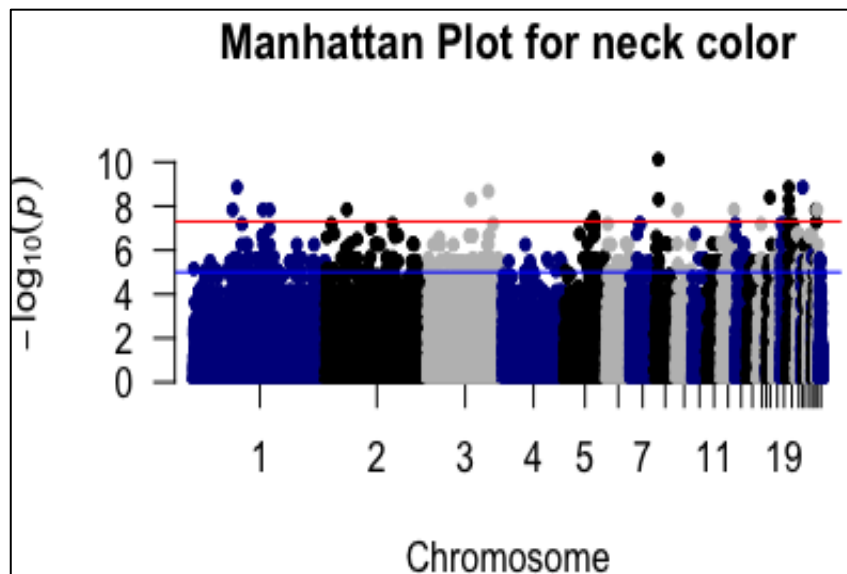


Figure 2. Manhattan plot for Brown-black neck color

The y-axis indicates the $-\log_{10}$ (P-values) of the genome-wide SNP in each GWAS meta-analysis. The blue horizontal lines represent suggestive lines ($-\log_{10}(1e-05)$, at $p < 0.005$) and red line represent the Bonferroni-corrected genome-wide significance (at $-\log_{10}(5e-08)$ at $p < 0.001$)

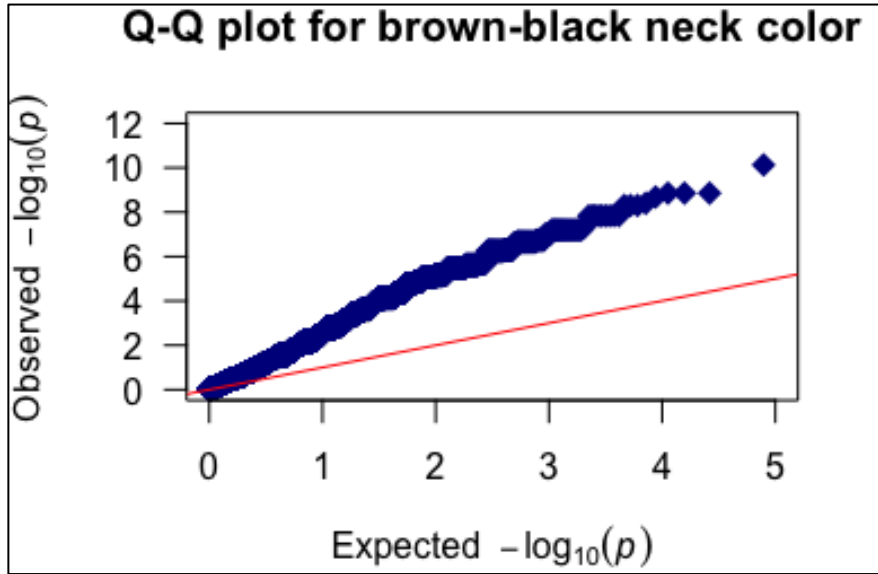


Figure 3. QQ plot for brown-black neck color with inflation factor ($\lambda=3.26639$)

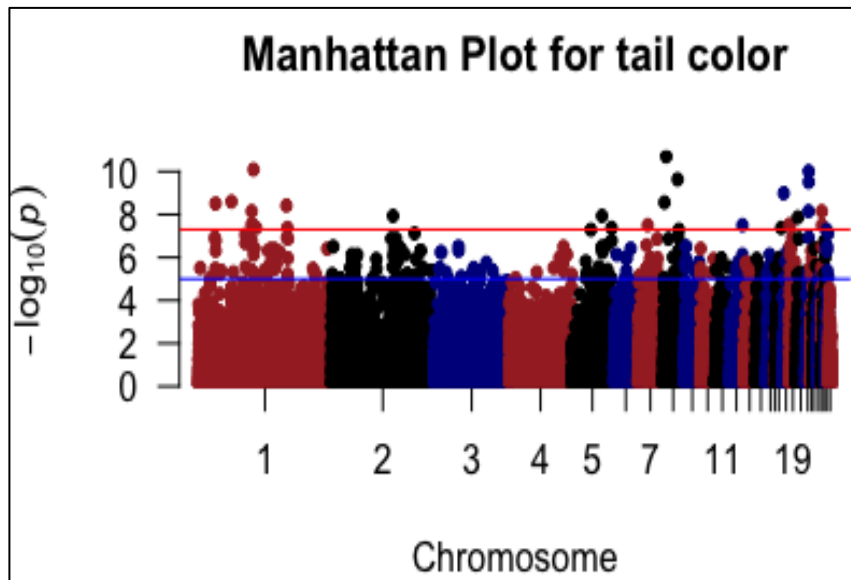


Figure 4. Manhattan plot for black-tail color

The y-axis indicates the $-\log_{10}(P\text{-values})$ of the genome-wide SNP in each GWAS meta-analysis. The blue horizontal lines represent suggestive lines ($-\log_{10}(1e-05)$, at $p < 0.005$) and red line represent the Bonferroni-corrected genome-wide significance (at $-\log_{10}(5e-08)$ at $p < 0.001$)

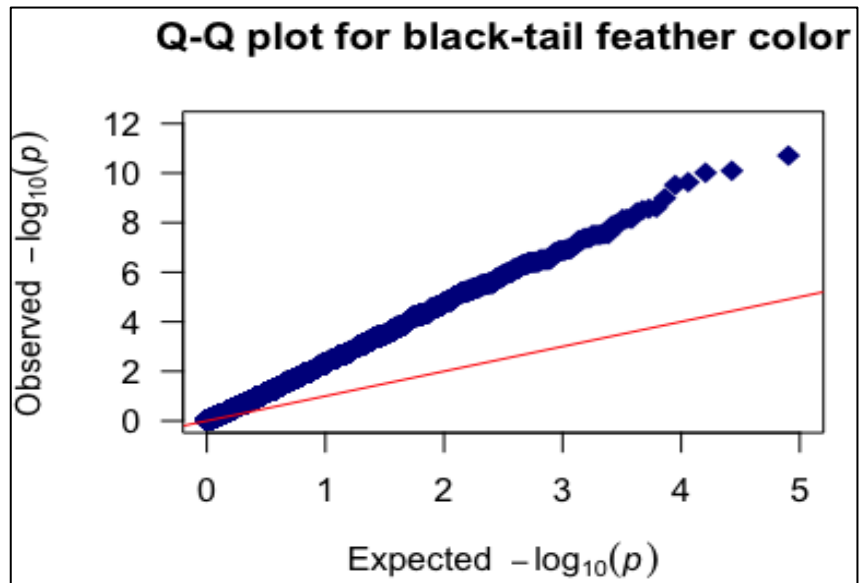


Figure 5. *QQ* plot for black-tail color with inflation factor ($\lambda=2.39234$)

Appendix 3: Principal component analysis

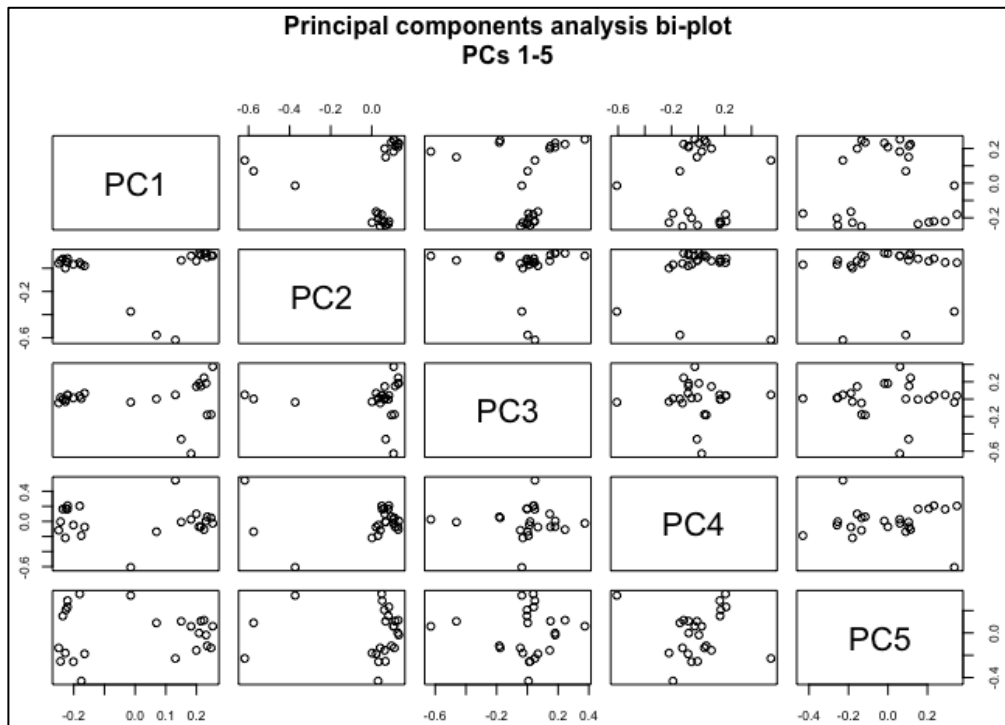


Figure 1. Bi-plot principal component (PCs) analysis for Gotlands breed